Original article

Cerebrospinal fluid ferritin in HIV infected patients with acute neurological episodes

F Deisenhammer, R F Miller, N S Brink, M J G Harrison, E J Thompson

Objectives: To measure cerebrospinal fluid (CSF) ferritin in HIV infected patients with acute neurological episodes and to correlate the findings with the type and severity of neurological disease.
Methods: CSF ferritin and the ratio of CSF to serum albumin (QAlb) were prospectively measured in 27 consecutive HIV infected patients admitted to a specialist unit for investigation of acute neurological episodes; the results were compared with their clinical diagnoses.
Results: Ten patients had HIV associated dementia complex, six had cryptococcal meningitis, two had primary CNS lymphoma and nine had miscellaneous conditions including herpes simplex virus encephalitis, cytomegalovirus encephalitis, cerebral toxoplasmosis and mononeuritis multiplex. Overall, 16 (59%) patients had raised CSF ferritin levels, ranging from 13·0 to 50·2 μg/l, (median = 16·1 μg/l: normal range = 1·0–12·0 μg/l). Thirteen of the 16 also had normal QAlb values, implying an intact CSF-blood barrier, and thus that local synthesis of ferritin had occurred. Elevated ferritin levels were not associated with particular neurological diagnoses. In those with HIV associated dementia complex there was no correlation between CSF ferritin levels and the severity of clinical cognitive deficit or the extent of magnetic resonance imaging abnormalities.
Conclusions: An elevated CSF ferritin level is a non-specific finding in HIV infected patients presenting with acute neurological episodes.
(*Genitourin Med* 1997;73:181–183)

Keywords: ferritin; AIDS; cerebrospinal fluid

Department of Clinical Neurochemistry, Institute of Neurology, The National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG
F Deisenhammer
E J Thompson

Department of Sexually Transmitted Diseases
R F Miller

Department of Virology, Division of Pathology and Infectious Diseases
N S Brink

Department of Neurology, University College London Medical School, Middlesex Hospital Site, London W1N 8AA
M J G Harrison

Correspondence to:
Dr R F Miller, Division of Pathology and Infectious Diseases, University College London Medical School, 4th Floor, The Mortimer Market Centre, Mortimer Market, off Capper Street, London WC1E 6AU, UK.

Accepted for publication
4 December 1996

Introduction

Ferritin is an iron storage protein which is present in virtually all body tissues. The highest concentrations of tissue ferritin are found in the liver and the reticuloendothelial system.[1] Ferritin has also been detected extracellularly in serum, urine, saliva and cerebrospinal fluid (CSF). In normal individuals CSF ferritin concentrations are approximately 10% of those in serum. Elevated CSF ferritin levels have been reported in a variety of neurological disorders including subarachnoid haemorrhage, intraparenchymal cerebrovascular events such as haemorrhage and infarction, bacterial and fungal meningitis, viral encephalitis and cerebral vasculitis.[2–6]

It has been suggested that the major source of CSF ferritin is either from local synthesis in microglial cells, due to the presence of red cell iron or iron containing haem compounds, or alternatively that it reflects necrotic changes in the brain causing release of cellular iron, such as mitochondrial cytochromes. Thus CSF ferritin has been considered a marker of microglial activation.[7 8] This study was undertaken to evaluate CSF ferritin levels in HIV infected patients presenting with acute neurological episodes. We sought to identify whether there was any correlation between elevated CSF ferritin levels and specific types of neurological disease in this patient group, or the severity of CNS changes.

Methods

We prospectively studied 27 consecutive HIV-1 antibody positive patients admitted to a specialist HIV/AIDS in patient unit at University College London Hospitals for investigation of neurological episodes. The study was carried out within the guidelines of the Middlesex Hospital Clinical Investigations panel.

Twenty four patients were homosexual males (23 white, one Indian) and three were heterosexuals—two were females (one of whom was of African origin, the other white) and one was an African male. All were profoundly immunosuppressed with CD4 + lymphocyte counts ranging from 0 to 0·22 × 10^9/l (median count = 0·05 × 10^9/l). Normal range = 0·35 to 2·20 × 10^9/l. Most patients had prior AIDS defining illnesses.

All patients were under the care of a specialist HIV physician (RFM) and were also seen by a neurologist (MJGH). They were investigated using a unit protocol. Following clinical assessment and magnetic resonance (MR) imaging of the brain lumbar puncture was carried out before institution of specific treatment. CSF protein and glucose concentrations were determined and the presence or absence of CSF pleocytosis was noted. CSF was stained histochemically and cultured for bacteria, mycobacteria and fungi and assayed for antibodies to *Treponema pallidum* and *Toxoplasma gondii* and for *Cryptococcus neoformans* antigen. An aliquot of CSF was analysed for the presence of cytomegalovirus (CMV), varicella zoster virus (VZV), herpes simplex virus type 1 (HSV-1) and Epstein–Barr virus (EBV) DNA by nested polymerase chain reaction (PCR) amplification, as previously described.[9–12]

Ferritin
A further aliquot of CSF was used to measure CSF ferritin using an ELISA assay as previously described. A value of $\geqslant$ 12 μg/l was considered to be elevated.[7]

Albumin
Measurement of albumin by electroimmunoassay was performed on CSF and serum obtained at the same time as lumbar puncture.[7] The ratio of albumin in CSF and serum was expressed as the ratio QAlb calculated from (CSF albumin/serum albumin) $\times$ 1000. A value of $\geqslant$ 8 was taken to reflect damage to the barrier between blood and brain.[13]

A diagnosis of HIV associated dementia complex was made on the basis of presentation with a subacute onset, over more than 1 month, of cognitive deficit, with decline in motivation, emotional control or change in social behaviour in the absence of clouding of consciousness, and the absence of demonstrable infection in the CSF by staining and bacterial, mycobacterial and fungal culture.[14] In addition no evidence of CMV, HSV-1, VZV or EBV infection could be demonstrated using molecular amplification techniques. In those with HIV associated dementia complex we recorded the severity of the clinical cognitive deficit using the Memorial Sloan-Kettering criteria,[15] and the MR imaging abnormalities of T-2 weighted diffuse white matter hyperintensity and cortical atrophy, as previously described.[16]

Cryptococcus neoformans meningitis was diagnosed by demonstration of the organism in CSF by staining and culture. Primary CNS lymphoma was diagnosed in two patients on the basis of typical MR appearances and demonstration of EBV DNA in CSF. Necropsy in one of these two patients and the patient with cerebral involvement with Hodgkin's disease also confirmed the diagnosis. The diagnosis of herpes simplex encephalitis was made at necropsy by the presence of extensive haemorrhagic necroses in the temporal lobes and by in situ hybridisation. The diagnosis of CMV mononeuritis multiplex was made by the typical clinical presentation, identification of CMV DNA in CSF, and objective response to anti-CMV therapy (ganciclovir).

Results

Results of routine CSF analysis and PCR amplification, ferritin and QAlb estimations are given in table 1. There was a striking lack of inflammatory response in the CSF of this patient group; only two had a pleocytosis (due to lymphocytes in both patients). Fifteen had normal CSF total protein results (normal range = 0·05 to 0·45 g/l) and only six had total protein values > 0·7 g/l; of these patients two had *C neoformans* meningitis and two had primary CNS lymphoma.

Four patterns of abnormality of CSF ferritin and QAlb were observed. Thirteen patients, including six with HIV associated dementia complex, had elevated CSF ferritin levels and normal values of QAlb. Three patients had both elevated ferritin levels and an increase in QAlb. Ten had normal CSF ferritin and QAlb levels including four patients with HIV associated dementia complex. One patient with primary CNS lymphoma had an elevated

Table 1 Diagnosis, CSF findings and ferritin levels in HIV infected patients

Diagnosis/ patient No	CSF findings: Cells (/mm³)	Protein (g/l)	PCR	Ferritin (µg/l)	QAlb
HIV associated dementia complex					
1	0	0·56	–	14·7	3·3
2	0	0·45	–	7·2	6·7
3	0	0·24	–	15·3	4·5
4	4	0·51	–	32·0	7·1
5	0	0·13	–	8·6	2·3
6	1	0·27	–	50·2	4·5
7	1	0·47	–	3·6	4·5
8	2	0·64	–	16·6	5·3
9	0	0·19	–	21·9	3·0
10	4	0·74	–	5·6	6·0
Cryptococcal meningitis					
11*	0	0·81	CMV DNA+	13·0	9·5
12	65	0·24	–	30·4	4·0
13	0	0·37	–	6·7	3·4
14	4	0·31	–	13·4	3·3
15	0	0·77	–	6·5	6·5
16	1	0·35	–	15·5	2·8
Cerebral lymphoma					
17	0	1·09	EBV DNA+	11·9	8·9
18	0	0·87	EBV DNA+	39·9	4·5
Miscellaneous conditions					
19 HSV encephalitis	0	0·37	–	13·0	7·1
20 CMV encephalitis/ CMV retinitis	0	0·87	CMV DNA+	15·7	17·4
21 CMV mononeuritis multiplex/retinitis	0	0·20	CMV DNA+	28·6	4·7
22 HIV mononeuritis multiplex	8	0·32	–	6·8	4·1
23 Vacuolar myelopathy	28	1·09	–	26·5	14·8
24 Cerebral toxoplasmosis	0	0·29	–	6·2	3·0
25 III N palsy	2	0·45	VZV DNA+	14·9	5·2
26 Cerebral Hodgkin's disease	0	0·29	–	8·2	3·8
27 Self limiting headache	0	0·48	–	2·8	3·4

CMV = cytomegalovirus; EBV = Epstein–Barr virus; VZV = varicella zoster virus; HSV = herpes simplex virus.
*Patient also had CMV retinitis; – = negative.

Table 2 Severity of dementia, MR imaging abnormalities and CSF ferritin levels in 10 patients with HIV associated dementia complex

Patient No	Severity of dementia*	MRI imaging abnormality†		CSF ferritin (μg/l)
		Atrophy	Diffuse white matter signal abnormalities	
1	Severe	Nil	Severe	14·7
2	Mild	Moderate	Nil	7·2
3	Moderate	Mild	Mild	15·3
4	Mild	Moderate	Mild	32·0
5	Mild	Moderate	Nil	8·6
6	Moderate	Moderate	Mild	50·2
7	Moderate	Mild	Mild	3·6
8	Moderate	Mild	Nil	16·6
9	Moderate	Severe	Severe	21·9
10	Mild	Nil	Mild	5·6

*Memorial Sloan-Ketting criteria (see Price and Brew).[15]
†Paley *et al.*[16]

QAlb but a normal ferritin level. Abnormal ferritin levels were not restricted to specific neurological diagnoses.

In those patients with HIV associated dementia complex there was no correlation between the level of CSF ferritin and the severity of clinical cognitive deficit, or the degree of cortical atrophy and the extent of diffuse white matter hyperintensity seen on T2 weighted MR imaging (table 2). All patients survived their episode except the two with primary CNS lymphoma, one with *C neoformans* meningitis (No 12) and the patients with CMV encephalitis and retinitis, cerebral Hodgkin's disease, and herpes simplex encephalitis. CSF ferritin levels were elevated in four of the six who died and in 12 of the 21 who survived.

Discussion

In this prospective study we sought to evaluate CSF ferritin levels as a marker of microglial activation and to correlate the findings with clinical disease in a consecutive series of HIV infected individuals undergoing investigation for acute neurological episodes. We found that 16 of our 27 patients (59%) had elevated CSF ferritin levels including those with a variety of diagnoses known to produce cerebral necrosis, for example, viral encephalitis due to HSV-1 and CMV. Surprisingly, other patients with conditions that also produce necrosis, including cerebral toxoplasmosis and primary CNS lymphoma, had normal CSF ferritin levels. We postulated that perhaps in those patients with HIV associated dementia complex the severity of the dementia measured by simple clinical and MR imaging criteria might correlate with CSF ferritin levels but we were unable to demonstrate any such association. It is tempting to suggest that the six patients with HIV associated dementia complex and elevated CSF ferritin levels neuropathologically might have a more encephalitic process. However, we are unable to confirm this possibility as we do not have necropsy data on this subgroup of patients.

As noted in previous reports of HIV infected individuals undergoing investigation of neurological episodes[10] there was a striking lack of inflammatory response in the CSF of our patients, the majority having no pleocytosis and normal QAlb ratios: over half had normal total protein levels. It was in this context that we observed elevations of CSF ferritin. Clearly local synthesis of ferritin rather than ferritin derived from plasma is the explanation for the finding of elevated CSF ferritin levels.[7] Even in those patients with elevated CSF ferritin the levels observed are somewhat lower than those previously reported in immune competent patients with neurological disease,[2 7] suggesting that although microglia are activated by HIV[17] their ability to synthesise ferritin is attenuated.

In conclusion these data show that an elevated CSF ferritin level is a non-specific finding in HIV infected patients presenting with acute neurological episodes, indicating that microglial activation is common in diverse clinical situations. Although elevated CSF ferritin levels were observed in some patients with conditions associated with cerebral necrosis the finding does not delineate the underlying pathology.

We thank Jane Rutherford for typing the manuscript.

1 Worwood M, Ahearne W, Dawkins S, Jacobs A. The characteristics of ferritin from human tissues, serum and blood cells. *Clin Sci Med* 1975;**48**:441–51.
2 Campbell DR, Skikne BS, Cook JD. Cerebrospinal fluid ferritin levels in screening for meningism. *Arch Neurol* 1986;**43**:1257–60.
3 Hällgren R, Terent A, Wide L, Bergström K, Birgegård G. Cerebrospinal fluid ferritin in patients with cerebral infarction or bleeding. *Acta Neurol Scand* 1980;**61**: 384–92.
4 Sindic CJM, Collet-Cassart D, Cambiaso CL, Masson PL, Laterre EC. The clinical relevance of ferritin concentration in the cerebrospinal fluid. *J Neurol Neurosurg Psychiatry* 1981;**44**:329–33.
5 Wick M, Fink W, Pfister P, Einhäupl K, Huber M, Fateh-Moghadam A. Ferritin in cerebrospinal fluid, differentiation between central nervous system haemorrhage and traumatic spinal puncture. *J Clin Pathol* 1988;**41**:809.
6 Zappone E, Bellotti V, Cazzola M, Ceroni M, Meloni F, Pedrazzdi P, *et al.* Cerebrospinal fluid ferritin in human disease. *Haematologica* 1986;**71**:103–7.
7 Keir G, Tasdemir N, Thompson EJ. Cerebrospinal fluid ferritin in brain necrosis: evidence for local synthesis. *Clin Chim Acta* 1993;**216**:153–66.
8 Koeppen AH, Borke RC, Dearborn RE, Hurwitz CG. Ferritin as a marker for normal and pathological microglia. *J Neurochem* 1991;**57 Suppl**:118C.
9 Aurelius E, Johansson B, Sköldenberg D, Forsgren M. Encephalitis in immunocompetent patients due to herpes simplex virus type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. *J Med Virol* 1993;**39**:179–86.
10 Fox JD, Brink NS, Zuckerman MA, Neild P, Gazzard BG, Tedder RS, *et al.* Detection of herpesvirus DNA by polymerase chain reaction in cerebrospinal fluid of human immunodeficiency virus-infected persons with neurologic disease: a prospective evaluation. *J Infect Dis* 1995;**172**:1087–90.
11 Grant AD, Fox JD, Brink NS, Miller RF. Detection of varicella-zoster virus DNA using the polymerase chain reaction in an immunocompromised patient with transverse myelitis secondary to herpes zoster. *Genitourin Med* 1993;**69**:273–5.
12 Mitchell SM, Fox JD, Tedder RS, Gazzard BG, Lightman S. Vitreous fluid sampling and viral genome detection for the diagnosis of viral retinitis in patients with AIDS. *J Med Virol* 1994;**43**:336–40.
13 Reiber H, Felgenhauer K. Protein transfer at the blood CSF barrier and the quantification of the humoral immune response within the CNS. *Clin Chim Acta* 1987;**16**:319–28.
14 Janssen RS, Cornblath DR, Epstein LG, Foa RP, McArthur JC, Price RW, *et al.* Nomenclature and research case definitions for neurological manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. *Neurology* 1991;**41**:778–85.
15 Price RW, Brew BJ. The AIDS dementia complex. *J Infect Dis* 1988;**158**:1079–83.
16 Paley MNJ, Chong WK, Wilkinson ID, Shepherd JK, Clews AM, Sweeney BJ, *et al.* Cerebrospinal fluid-intracranial volume ratio measurements in patients with HIV infection: CLASS image analysis technique. *Radiology* 1994;**190**:879–86.
17 Puilliam L, Herndier BG, Tang NM, McGrath MS. Human immunodeficiency virus-infected macrophages produce soluble factors that cause histological and neurochemical alterations in cultured human brains. *J Clin Invest* 1991;**87**:503–12.